REMARKS

The claims are 1-48 and 55. The claims have been amended to resolve minor informalities.

As requested, Fig. 15 has been amended to identify it now as Fig. 1. In addition, the specification has been amended to change the reference to Fig. 15 to read -- Fig. 1--. In addition, an earlier reference to Fig. 15 has been deleted.

The objections to claims 2, 3, 9 and 10 advanced at page 3 of the outstanding official action has been resolved by appropriate amendment of the claims.

The Examiner had objected to claims 1, 3, 6-10, 15, 17, 19 and 55 under Rule 112, first paragraph on pages 3-5 of the official action. Appropriate amendments to claims 3, 9, 10, 17 and 55 have been made to address each of the Examiner's stated concerns and it is requested that the objections thereto be withdrawn. The Examiner objected to claims 6, 7 and 19 on the ground that they claim a gene by its coding regions only. That objection is respectfully traversed.

In claims 6, 7 and 19, Applicants do not claim a naturally occurring gene, but claim a DNA fragment containing coding regions for TomL, TomM, TomN, TomO and TomT. Such a DNA fragment can be isolated from nature or can be an artificial or a recombinant fragment. The function of such a protein is defined solely by the region in coding the structure (the structural gene) and not by the non-transcription/non-translation regions, such as a promoter, terminator and spacer. In bacteria, particularly, genes are constituted as operons, which is a transcription unit comprised of a regulator gene and several structural genes. It is well known and understood that in such an operon each component is expressed with appropriate spacers. Thus, spacers are not essential and the

promoter can be heterologous. Accordingly, once coding regions are identified, then the coding regions can be arranged with any appropriate spacers and promoters to be expressed properly.

The Examiner had objected to claims 3, 7, 9, 10, 15, 17, 19 and 55 under Rule 112, first paragraph on pages 5-8 of the Official Action, as not being enabling for the moieties recited therein. Applicants have now provided appropriate amendments to such claims in order to resolve the enabling objections. In particular, appropriate coding regions have been recited. Withdrawal of the objections is respectfully requested.

On pages 8-10 of the outstanding action, claims 1 and 15 were rejected under Rule 112, first paragraph, on the ground that the deposit requirements have not been satisfied with respect thereto. That objection is respectfully traversed.

The Examiner has alleged the specification does not provide information regarding the dates of deposit, the name of microorganism and the address of the depository for Burkholderia cepacia KK01. The Examiner's attention is directed to specification page 9, lines 2-13 in which such information is provided.

A deposit of such microorganism has been made under the Budapest Treaty as noted on specification page 9. Applicants' undersigned attorney has provided an appropriate statement that the deposit of KK01 has been made and that the specific restrictions imposed by the depositor will be irrevocably removed upon the granting of the patent. Accordingly, withdrawal of the objection thereato is respectfully requested.

Claims 3, 4, 9, 10, 11, 15, 17 and 55 were rejected under Rule 112, second paragraph, as being indefinite as set forth on page 11 of the outstanding official action. That rejection is respectfully traversed.

Claims 3, 9, 10, 15, 17 and 55 have now been amended as appropriate in order to meet the concerns raised by the Examiner.

With regard to the "Response to Arguments" portion of the office action on page 12, with regard to the explanation of the relation between a vector and DNA fragment, Applicants submit that the term "vector" means a self-replicating DNA molecule which can carry a DNA segment or passenger into a host cell. See page 26, line 15 to page 27, line 1 and page 27, lines 20-27 of the specification for an explanation of a vector of a wide host range. The term "expression vector" means a self-replicating DNA molecule and a promoter for the expression of the passenger DNA. Specification page 59, lines 4-14 discloses an expression vector.

Thus, a recombinant vector includes at least a vector and a passenger DNA and an expression vector includes at least a vector, a passenger DNA and a promoter. Accordingly, it is believed that the language in claims 11 and 55 is appropriate.

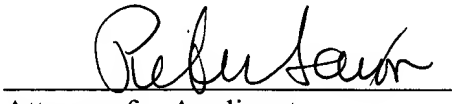
Applicants wish to provide an explanation of the amendment of claims 3, 9, 10, 17 and 55 with regard to the phrase "a sequence that hybridizes under stringent conditions to a hybridization probe." Kindly note that hybridization under stringent conditions excludes sequences of 70% homology. Accordingly, the sequence of Shields et al. is not included.

Applicants have endeavored to meet each of the objections raised by the Examiner and it is requested that the Amendment, Request for Approval of Proposed Drawing Changes and Notice re: Deposit of Microorganisms be entered. Further, it is respectfully requested that based on the above amendments and arguments that the final rejection be withdrawn, the claims allowed and the case passed to issue.

In any event, it is requested the amendment be entered because it complies with the suggestions advanced by the Examiner, resolves informalities and places the case in better form for possible appeal.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,



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Appendix

Application No. 09/430,029
Docket No.: 35.C13892

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO SPECIFICATION

The paragraph at page 21, lines 16 and 17 has been amended as follows:

--Fig. [15] 1 shows time-course changes in TCE in the gas phase in Example 3.--

The paragraph beginning at page 48, line 14 and ending at page 49, line 5 has been amended as follows:

--Eight brown colonies were found in these colonies and picked up. Recombinant plasmid DNA carrying toluene monooxygenase gene was extracted from the cells of each brown colony and the restriction map thereof was determined. It was found that all recombinant plasmids derived from the 8 colonies had a common insertion fragment of 5.8 kb. A plasmid containing only the common fragment of 5.8 kb was designated as pKKO1 and a restriction map of the inserted DNA fragment was made [(See Fig. 1)]. A recombinant E.coli HB101 carrying a plasmid containing a 8.5 kb insertion fragment containing this common 5.8 kb fragment was deposited in the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession No. FERM BP-6916. Its microbiological characteristics were identical to those of E.coli HB101 except that it can degrade aromatic compounds and chlorinated aliphatic hydrocarbon compounds.--

Appendix

Application No. 09/430,029
Docket No.: 35.C13892

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO

CLAIMS 2-4, 9-11, 15, 17 AND 55

--2. (Twice Amended) [The isolated] A DNA fragment[, said] isolated [DNA-fragment derived] from [Borkholderia] Burkholderia cepacia KK01 wherein the DNA fragment has a nucleotide sequence of SEQ ID NO: 1 [in the Sequence Listing].

3. (Twice Amended) An isolated DNA fragment having a nucleotide sequence that hybridizes under stringent conditions to a hybridization probe with a nucleotide sequence consisting of SEQ ID NO: 1 or a complement of SEQ ID NO: 1 [of SEQ ID NO: 1 with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration , or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids].

4. (Amended) A recombinant DNA comprising a vector enabling maintenance or replication in a host, [and] said vector including a DNA fragment according to any one of claims 1 to 3.

9. (Twice Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO:3 or a mutant thereof, a second sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1495..1761 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4 or a mutant thereof, a third sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1803..3350 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomN having an amino acid of SEQ ID NO: 5 or a mutant thereof, a fourth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3428..3781 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6 or a mutant thereof, and a fifth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3810..4871 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 or a mutant thereof, and the first to fifth sequences are aligned so that expressed polypeptides can form an active monooxygenance protein.

[wherein in at least one of the first to fifth sequences of the DNA fragment substitution with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between

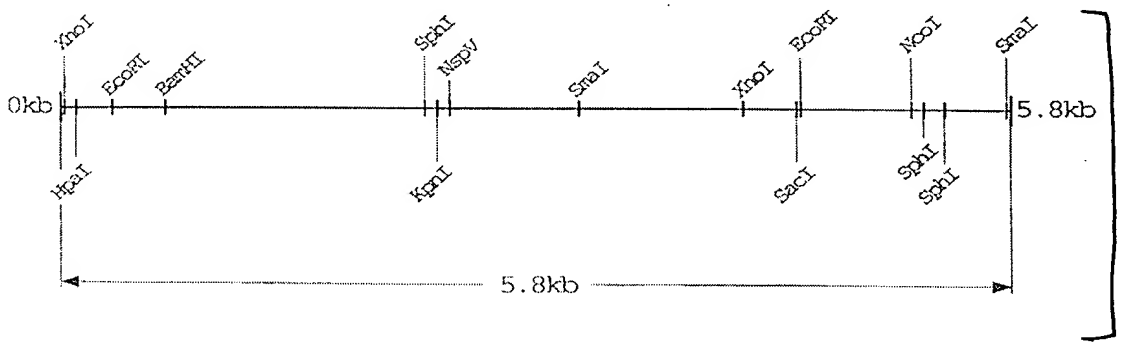
aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.]

10. (Twice amended) An isolated DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomK[, the polypeptide TomK] having an amino acid sequence of SEQ ID NO: 2, or a mutant thereof [with substitution of at least one nucleotide, said substitution resulting in 1) no amino acid change with code degeneration, or 2) amino acid substitution only between aliphatic amino acids, between sulfur-containing amino acids, between hydroxy amino acids, between aromatic amino acids, between basic amino acids, and between acidic amino acids.]

11. (Three Times Amended) A recombinant DNA comprising a vector, wherein said vector contains a promoter[, and the DNA fragment according to any one of claims 6, 7, or 9, wherein the vector and the promoter are] which is functionally ligated to [the] a DNA fragment according to any one of claims 6, 7 or 9 to enable expression of the toluene monooxygenase encoded by the DNA fragment.

15. (Twice Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, the recombinant DNA comprising a vector enabling maintenance or replication in a host and a DNA fragment of about 5.8 Kb

containing a toluene monooxygenase gene having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, [and having a restriction map of DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene having 1 BAmHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of:



said DNA-fragment derived from [Borkholderia] Burkholderia cepacia KK01.

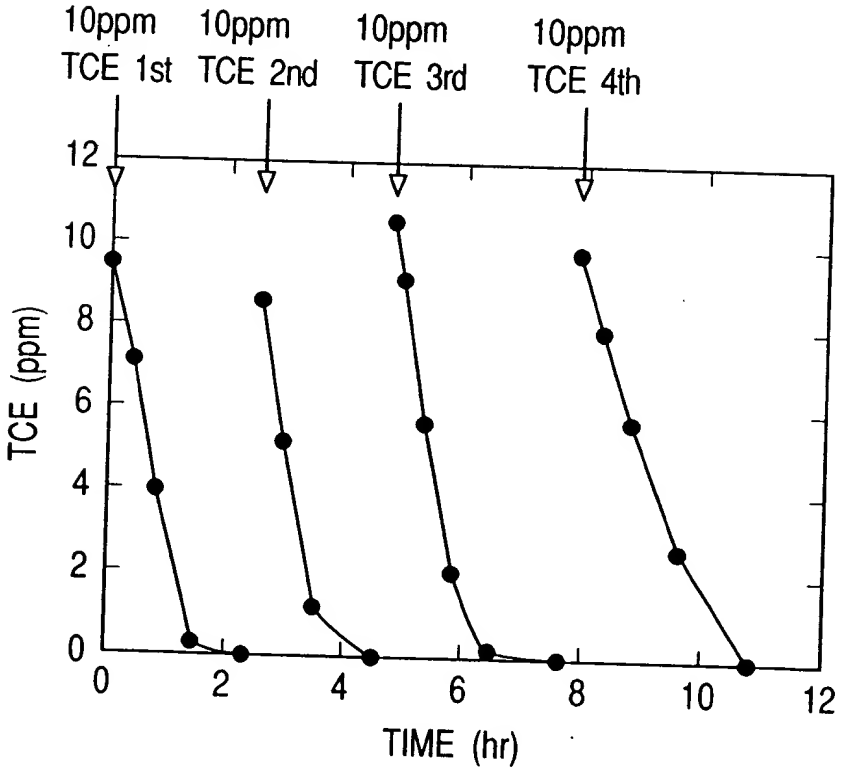
17. (Twice Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, where the recombinant DNA comprises a vector enabling maintenance or replication in a host, said vector including [and] a DNA fragment ligated thereto having a sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of SEQ ID NO: 1 or a complement of SEQ ID NO: 1 and [of the Sequence Listing with deletion, substitution and/or addition of one or more nucleotides, still] encoding an active toluene monooxygenase, wherein the DNA fragment [has] contains a toluene monooxygenase region of 4.9 kb or less.

55. (Three Times Amended) A recombinant DNA comprising a vector, a promoter, a first DNA fragment being the DNA fragment of any one of claims 6, 7 or 9, and a second DNA fragment, said second DNA fragment comprising a region encoding a polypeptide TomK having an amino acid sequence of SEQ ID NO: 2[,] and a property to enhance the toluene monooxygenase activity of a protein comprised of at least TomL to TomP; or a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding [a variety of] TomK or an active mutant thereof, [in which the amino acid sequence of SEQ ID no: 2 is altered with the proviso that the property to enhance the toluene monooxygenase activity is not impaired,] wherein the first DNA fragment [has] containing a toluene monooxygenase encoding region of 4.9 kb or less is

functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.--

1

FIG. ~~15~~


10ppm TCE 1st
10ppm TCE 2nd
10ppm TCE 3rd
10ppm TCE 4th
TCE (ppm)
TIME (hr)
0
2
4
6
8
10
12